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Effects of Different Dietary Levels of Mannanoligosaccharide on Growth Performance and Gut Development of Broiler Chickens

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ABSTRACT : Different levels of dietary mannanoligosaccharide (Bio-MOS, Alltech Inc.) were evaluated for their efficacy on performance and gut development of broiler chickens during a 6-week experimental period. Experimental diets contained (g MOS/kg diet) a low (0.5 g during the entire period), medium (1 g during the entire period), high (2 g during the entire period), or step down (2 g in the first week; 1 g in the second and third week; 0.5 g in the last three weeks) level of MOS. Control diets included a negative and a positive control (zinc bacitracin, ZnB, 50 ppm and 30 ppm in the first and last three weeks, respectively). MOS supplementation improved the growth performance of young birds and the effects became less when the birds got older. The growth response of birds was more obvious at the high dosage level of MOS treatment than the other MOS treatments and the growth performance of birds fed on the high MOS diet was comparable to that of birds fed on the ZnB diet. Depending on the dosage level and the age of birds, MOS seemed to reduce the size of the liver and the relative length of the small intestine but did not affect the relative weight of the other visceral organs (proventriculus, gizzard, pancreas, bursa and spleen) and that of the small intestine. A numerical increase in the small intestine digestibility of nutrients was noticed in the young birds fed on the MOS diet(s), but not in the older ones. Medium and/or high MOS treatment also increased the villus height of the small intestine of birds at different ages. Similar results were observed on the ZnB treatment. However, MOS and ZnB affected caecal VFA profile in different ways. MOS increased, or tended to increase, whereas ZnB reduced individual VFA concentrations in the caeca. (**Key Words** : Mannanoligosaccharide, Bio-MOS, Performance, Intestinal Digestibility of Nutrient, Gut Morphology, Cecal VFA Profile, Broiler Chickens)

INTRODUCTION

The reality that the use of antibiotic growth promoters is being curtailed by legislative and market actions has led to a new urgency in the search for replacements (Dibner and Richards, 2005). One of the possible alternatives to antibiotic growth promoters has been shown to be mannanoligosaccharide (MOS), derived from the outer cell wall of *Saccharomcyes cerevisiae*. It contains phosphorylated mannans, glucans and some protein intermixed. The addition of MOS to broiler chicken diets was reported to have positive effects on growth performance (Hooge, 2003; Rosen, 2006) but the supplementing levels of MOS varied by trials and by feed phase in different studies, ranging from 0.5 g MOS/kg diet to 5 g MOS/kg diet. In commercial operations MOS is used in small doses (0.5 to 2 g/kg) for economic reasons. The dose-response studies of MOS showed that the optimal dosage for growth performance is around 2 g MOS/kg diet (Kuprecht and Zobac, 1997; Tucker et al., 2003).

Studies on the effects of MOS on the gut development and physiology of broiler chickens are limited. Iji et al. (2001) examined the effects of MOS (0, 1, 3 and 5 g MOS/kg diet) on the intestinal structure and function of birds during a 21-day feeding period. Improvements in the intestinal structure and function were noticed in birds supplemented with medium or high level of MOS but the effects of MOS on the growth performance were minimal.

In considering the possibility of total antibiotic restrictions in the future and potential benefits of MOS in poultry feeding, the effects of MOS on the growth as well as gut development and function of birds need to be explored. The present study was designed to compare the effects of different dietary MOS levels on the growth performance of broiler chickens with a positive control (zinc bacitracin) and a negative control. Intestinal nutrient digestibility, the general morphology of gastrointestinal

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Table 1. Composition and nutritive value of basal diet

Component	Feeding period (wk)	
	1-3	4-6
Wheat (g/kg)	50.00	50.00
Corn (g/kg)	585.00	645.00
Soybean meal (g/kg)	250.00	195.00
Meat meal (g/kg)	80.00	85.00
Limestone (g/kg)	7.30	3.40
Lysine-HCl (g/kg)	3.00	3.00
DL-methionine (g/kg)	3.00	2.50
Salt (g/kg)	0.70	0.30
Sodium bicarbonate (g/kg)	4.50	3.50
Choline chloride (g/kg)	3.00	1.00
Vegetable oil (g/kg)	11.00	9.00
L-threonine (g/kg)	0.50	0.30
Vitamin and mineral premix[1] (g/kg)	2.00	2.00
Calculated chemical composition		
ME (MJ/kg)	12.56	12.75
Crude protein (g/kg)	220	200
Crude fiber (g/kg)	28	26.6
Crude fat (g/kg)	45	46
Lys (g/kg)	13	11.8
Met+cys (g/kg)	9.7	8.9
Ca (g/kg)	10.3	9.5
P available (g/kg)	5.1	4.8
Na (g/kg)	1.9	1.8
Cl (g/kg)	2.2	2.4

[1] Supplied per kg of diet (mg): vitamin A (as *all-trans* retinol), 12,000 IU; cholecalciferol, 3,500 IU; vitamin E (as d-α-tocopherol), 44.7 IU; vitamin K_3, 2 mg; thiamine, 2 mg; riboflavin, 6 mg; pyridoxine hydrochloride, 5 mg; vitamin B_{12}, 0.2 mg; biotin, 0.1 mg; niacin, 50 mg; D-calcium pantothenate, 12 mg; folic acid, 2 mg; Mn, 80 mg; Fe, 60 mg; Cu, 8 mg; I, 1 mg; Co, 0.3 mg; and Mo, 1 mg.

tract (GIT), the histology of small intestinal mucosa, and caecal volatile fatty acid (VFA) concentrations of chicken were chosen to examine the effects of MOS on the nutrient utilization and gut development of birds which might explain the growth response of birds induced by the addition of MOS.

MATERIALS AND METHODS

Experimental design and diets

The basal diets, consisting mainly of corn, wheat and soybean meal, were used in a 2-phase feeding programme with the starter feed provided from 1 to 3 weeks of age and the finisher diet from 4 to 6 weeks (Table 1). Experimental diets contained (g Bio-MOS/kg diet) a low (0.5 g during the entire period), medium (1 g during the entire period), high (2 g during the entire period), or step down (2 g in the first week; 1 g in the second and third week; 0.5 g in the last three weeks) MOS treatment. Control diets included a negative (NC) and a positive control (zinc bacitracin, ZnB, 50 ppm and 30 ppm in the first and last three weeks, respectively). A commercial xylanase product (Allzyme PT, Alltech Pty Ltd. Australia) was included in the basal diet at the recommended level (0.5 g/kg feed). Titanium dioxide, 5 g/kg, was incorporated in the basal diet as a marker for the calculation of digestibility coefficients.

Bird management

Three hundred and eighty-four one-day-old male Cobb broiler chickens (42.6 g±3.55) were obtained from a local hatchery and randomly assigned to 48 cages in four-tier battery brooders housed in an environmentally controlled room. Each of the 6 dietary treatments was randomly assigned to 8 cages (600×420×230 cm) with 8 birds per cage. The birds were transferred to slide-in cages (800×740×460 cm) in an environmentally controlled room at the end of week 3. Room temperature was at 34±1°C on the first day and gradually reduced to 24°C by the end of the third week. The lighting program was 18 h light and 6 h darkness throughout the trial.

The experiment complied with the guidelines of the University of New England with respect to animal experimentation and care of chickens under study.

Growth performance and AME measurement

Body weight and feed intake (FI) were recorded on a cage basis at weekly intervals. Feed was offered *ad libitum* and water was freely available at all times during the 42-d trial period. Mortality was recorded daily and feed per gain values were corrected for mortality.

Total collection of excreta was carried out for the determination of AME. Feed intake and excreta output were measured quantitatively per cage over 4 consecutive days (d 28 to d 32). Excreta were pooled within each cage, and dried in an oven kept at 80°C. Dried samples were ground to pass through a 0.5 mm screen and stored in airtight plastic containers until further chemical analysis. The AME values were calculated using the following formula. Appropriate corrections were made for differences in dry matter content.

$$AME = ((FI \times GE_{diet}) - (Excreta\ output \times GE_{excreta}))/FI$$

Collection and analysis of animal samples

At 7, 21 and 42 days of age, one bird per cage (8 birds per treatment) was killed by cervical dislocation, and the bowels were excised. The proventriculus and gizzard were emptied and weighed. The small intestine was divided into three segments: duodenum (from gizzard outlet to the end of the pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the caecal junction). Approximately 2 cm length of the proximal jejunum and proximal ileum were removed for gut morphological measurements. The gut samples were flushed with ice-cold phosphate-buffered

saline (PBS) at pH 7.4 and immediately placed in 10% formalin solution.

Each intestinal segment was emptied by gentle pressure and the length and weight were recorded, as was the body weight of the birds they were excised from and thus calculated the relative length and weight of the segment. The weight of pancreas, liver, spleen, and bursa was also monitored. The contents of the jejunum and ileum were collected, freeze-dried, and milled (0.5 mm screen); the contents of cecum were stored at -20°C until analysis was performed.

Dry matter, gross energy, protein and starch : The dry matter content was determined gravimetrically following drying at 105°C for 24 h (for diets) or drying at 80°C for 48 h (for excreta). Gross energy (GE) of the feed and digesta was determined using a bomb calorimeter (Calorimeter C7000, Protech group, Australia) standardised with benzoic acid. The nitrogen contents were determined (Leco FP-2000 analyzer) and the protein contents were calculated using a multiplication factor of 6.25. Starch was determined as glucose using a glucose oxidase and peroxidase method (GPD-Perid kit supplied by Boehringer-Mannheim Australia, Castle Hill, NSW, Australia).

Digestibility : Titanium dioxide in the feed and digesta was determined by the method of Short et al. (1996). Dried digesta, 0.1 g, was ashed and dissolved in 7.4 M sulphuric acid. Hydrogen peroxide (30% vol.) was subsequently added, resulting in the typical orange colour, the intensity of which is dependent upon the titanium concentration. Aliquots of the solutions obtained and of similarly prepared standard solutions were analysed using a UV spectrophotometer (Model 50-120, Hitachi, Japan) and measuring the absorbance at 410 nm. Small intestinal digestibility coefficient of starch and protein was then estimated using the formula:

DC = 1-[(digesta nutrient (g/kg)/ digesta TiO_2 (g/kg)) /(diet nutrient (g/kg)/diet TiO_2 (g/kg))]

Histology analysis : Tissue slices, 1-2 mm thick, were cut from the gut samples from each section, enclosed in a plastic tissue cassette then processed over a 19 h period in an automatic tissue processor. Processing involved serial dehydration with ethanol, clearance with histolene, then impregnation with wax. The tissue was embedded in paraffin wax prior to sectioning on a microtome. Separate 7 μm sections were placed on a glass slide for staining with haematoxylin and counter-staining with eosin, then mounted in a DPX medium (Fluka Cheme, Buchs, Australia). The images were captured at 4 × and 5 × magnification using a Leica DLMB Microscope in bright field mode and a Spot RT digital camera. The images were stored and the Spot software was used to calibrate and then measure the villus height and crypt depth. About 15 villi and 15 crypts were measured in each type of tissue from each chicken.

VFA analysis : Concentrations of acetate, propionate, and butyrate were determined by gas chromatography (GC, Model CP 3800, Varian Australia, Frenches Forst, Australia). Approximately 2 to 3 g of thawed digesta were suspended in 3 ml of 0.1 M sulphuric acid in a screw-capped tube, centrifuged (15 min at 12,000×g) at 4°C. One tenth ml caproic acid were added to 1 ml of the supernatant, which were transferred into Thundberg tube. After the sample was frozen in liquid nitrogen, and the air sucked out, it was bathed in thermos full of liquid nitrogen over night. Subsequently, the sublimated sample was thawed for analysis by GC. The GC was equipped with a flame ionization detector and a polyethylene glycol packed column (1.5 m long, 5.6 mm). The column was operated at 100 to 150°C with high purity helium, at 20 ml/min, as the

Table 2. Feed intake, body weight gain (BWG), feed:gain ratio, energy utilization and mortality of birds fed on the experimental diets[1]

	NC	0.5 g/kg MOS	1 g/kg MOS	2 g/kg MOS	Stepdown MOS	ZnB	SEM
1-3 wk							
Feed intake (g/bird)	1,160	1,208	1,167	1,203	1,141	1,169	33.8
BWG (g/bird)	826^{b}	872^{ab}	835^{ab}	877^{a}	836^{ab}	847^{ab}	19.2
Feed:gain ratio	1.41	1.39	1.40	1.37	1.37	1.38	0.022
4-6 wk							
Feed intake (g/bird)	3,465	3,437	3,439	3,405	3,367	3,482	80.5
BWG (g/bird)	1,936^{ab}	1,918^{ab}	1,923^{ab}	1,910^{ab}	18,94^{b}	1,995^{a}	31.1
Feed:gain ratio	1.79^{a}	1.79^{a}	1.79^{a}	1.78^{ab}	1.78^{ab}	1.75^{b}	0.015
1-6 wk							
Feed intake (g/bird)	4,626	4,646	4,606	4,609	4,509	4,651	66.7
BWG (g/bird)	2,762^{ab}	2,790^{ab}	2,758^{ab}	2,787^{ab}	2,730^{b}	2,842^{a}	31.7
Feed:gain ratio	1.68^{a}	1.67^{ab}	1.67^{a}	1.65^{ab}	1.65^{ab}	1.64^{b}	0.013
AME (MJ/kg DM)	14.18	14.70	14.13	14.45	14.36	14.56	0.44
Mortality (%)	1.56	6.25	3.13	6.25	6.25	6.25	-

[1] Six experimental diets were included: 0.5 g MOS/kg diet; 1 g MOS/kg diet, 2 g MOS/kg diet, Step down MOS diet (2 g in the first week; 1 g in the second and third week; 0.5 g in the last three weeks), a negative control diet (NC), and a positive control (zinc bacitracin, ZnB, 50 ppm and 30 ppm in the first and last three weeks, respectively) diet.

[a,b] Means within the same row with no common superscript differ significantly ($p<0.05$).

Table 3. Effects of dietary treatments [1] on the relative weight (%BW) of visceral organ of broiler chickens on days 7, 21 and 42

	NC	0.5 g/kg MOS	1 g/kg MOS	2 g/kg MOS	Stepdown MOS	ZnB	SEM
Day 7							
Proventriculus	1.09	1.04	0.97	0.97	1.09	1.01	0.06
Gizzard	4.52	4.00	4.23	4.02	4.11	4.17	0.19
Pancreas	0.53	0.45	0.52	0.48	0.53	0.52	0.05
Liver	5.19[a]	4.32[b]	4.51[ab]	4.43[ab]	4.24[b]	4.67[ab]	0.25
Spleen	0.10	0.10	0.10	0.10	0.09	0.09	0.02
Bursa	0.15	0.20	0.19	0.16	0.19	0.16	0.02
Day 21							
Proventriculus	0.45	0.46	0.46	0.45	0.43	0.43	0.03
Gizzard	2.03	2.07	2.16	2.12	2.10	2.05	0.11
Pancreas	0.25	0.25	0.27	0.26	0.26	0.24	0.02
Liver	3.01	2.94	3.09	3.06	2.88	2.82	0.22
Spleen	0.09	0.10	0.09	0.10	0.08	0.09	0.00
Bursa	0.23	0.23	0.26	0.26	0.24	0.22	0.03
Day 42							
Proventriculus	0.34	0.35	0.34	0.37	0.32	0.31	0.03
Gizzard	1.74	1.66	1.76	1.77	1.68	1.73	0.11
Pancreas	0.19	0.17	0.17	0.17	0.19	0.19	0.02
Liver	2.45	2.36	2.43	2.56	2.31	2.32	0.16
Spleen	0.11	0.11	0.12	0.12	0.12	0.12	0.02
Bursa	0.16	0.16	0.16	0.20	0.16	0.16	0.03

[1] See Table 2.

[a,b] Means within the same row with no common superscript differ significantly ($p<0.05$).

carrier gas.

Statistical analysis

The software package SPSS. (Version 12.0. SPSS Inc.), was used to perform the statistical analysis of data obtained in this study. Data were analysed by one way ANOVA. The differences among mean values were identified by the least significant difference. Differences among treatments were deemed to be significant only if the p-value was less than 0.05. All data were expressed as means.

RESULTS

Growth performance

In general. the supplementation of MOS to the basal diet improved the growth performance of birds compared to the negative control in the first three weeks but not in the last three weeks (Table 2). Although there was no linear relationship between the growth response and the increasing level of MOS, birds in the high MOS group showed better body weight gain (BWG) and feed:gain ratio (FCR) than birds in the other MOS groups.

A 6% increase ($p<0.05$) in BWG and a 2% decrease ($p>0.05$) in FCR were observed with birds in the high MOS group compared to the negative control in the first three weeks. These improvements were comparable to ZnB treatment (3% increase in BWG and 2% decrease in FCR). Feed intake of birds numerically increased on the high MOS treatment compared to the negative control during the same period.

No significant differences were observed in the growth performance of birds among MOS treatments and the negative control in the last three weeks. Birds fed on step-down MOS diet had lower ($p<0.05$) BWG than those fed on ZnB diet. ZnB supplementation reduced ($p<0.05$) FCR of birds compared to the negative control.

By the end of day 42. birds fed on ZnB diet had the highest body weight and the lowest FCR among all the dietary treatments. No significant differences were noticed in AME and mortality among dietary treatments.

Gross morphology of gastrointestinal tract

Only the relative weight of the liver and the relative length of the small intestine were affected by the addition of MOS and the effects were dependent on the age of birds as well as the dosage level of MOS.

At 7 days of age. low and medium MOS treatment significantly reduced ($p<0.05$) the relative weight of the liver whereas high and step-down MOS treatments numerically reduced the relative weight of the liver compared to the negative control (Table 3). A similar result was noticed with birds fed on ZnB diet.

At 21 days of age. the step-down MOS treatment reduced the relative length of duodenum compared to ZnB treatment (Table 4). At 42 days of age. birds fed on the medium MOS diet tended ($p<0.08$) to have shorter duodenum than controls. whereas step-down MOS reduced ($p<0.05$) the relative length of jejunum compared to the high MOS treatment. In addition. a shorter ileum was

Table 4. Effects of dietary treatments[1] on the relative weight (% BW) and relative length[2] (cm/% BW) of the small intestine of broiler chickens on days 7, 21 and 42

	NC	0.5 g/kg MOS	1 g/kg MOS	2 g/kg MOS	Stepdown MOS	ZnB	SEM
Relative weight (% BW)							
Day 7							
Duodenum	2.09	2.05	2.06	2.03	2.14	2.08	0.10
Jejunum	2.76	2.54	2.84	2.80	2.74	2.64	0.14
Ileum	1.70	1.75	1.63	1.64	1.81	1.66	0.16
Day 21							
Duodenum	0.88	0.90	0.91	0.95	0.88	0.79	0.05
Jejunum	1.50	1.44	1.44	1.40	1.45	1.33	0.10
Ileum	0.93	0.95	0.96	0.94	0.92	0.82	0.06
Day 42							
Duodenum	0.54	0.51	0.48	0.49	0.46	0.53	0.03
Jejunum	0.91	0.89	0.87	0.96	0.89	0.90	0.06
Ileum	0.66	0.66	0.67	0.68	0.69	0.65	0.04
Relative length (cm/% BW)							
Day 7							
Duodenum	11.30	11.26	11.66	10.72	11.60	11.67	0.72
Jejunum	26.23	25.43	26.82	25.90	27.76	25.83	1.72
Ileum	25.15	23.10	25.37	24.91	26.75	24.00	1.93
Day 21							
Duodenum	2.79^{ab}	2.98^{ab}	2.86^{ab}	2.76^{ab}	2.66^{b}	3.04^{a}	0.13
Jejunum	6.57	6.70	6.73	6.23	6.48	6.64	0.38
Ileum	5.71	6.18	6.27	5.77	5.95	6.00	0.36
Day 42							
Duodenum	1.16	1.17	1.06	1.08	1.08	1.17	0.05
Jejunum	2.60^{ab}	2.65^{ab}	2.62^{ab}	2.72^{a}	2.51^{b}	2.58^{ab}	0.06
Ileum	2.62^{bcd}	2.67^{bc}	2.35^{cd}	2.70^{b}	2.50^{acd}	2.46^{ad}	0.10

[1] See Table 2. [2] Relative length = the length of the segment (cm)/% BW.
[a,b] Means within the same row with no common superscript differ significantly ($p<0.05$).

noticed in birds fed ZnB diet compared to the negative control and some MOS treatment(s).

Intestinal digestibility of nutrient, morphological measurement and caecal VFA concentration

The effects of dietary treatments on the intestinal digestibility coffecient of nutrient are shown in Table 5. There were no significant differences in apparent jejunal and ileal digestibility of protein and starch with 7-day-old birds among dietary treatments..

No significant differences among dietary treatments were observed in the apparent digestibility of starch and protein at the jejunum or ileum with 21-day-old and 42-day-old birds. however. the small intestinal digestibility of protein was numerically higher in 21-day-old birds fed on the medium, high MOS or ZnB diet than those fed on the negative control diet.

Depending on the dosage level, the supplementation of MOS altered the gut morphology of birds (Table 6). Birds given medium or high MOS diet had longer ($p<0.05$) villi at the jejunum compared to those birds in the negative control or ZnB group at the end of weeks 1 and 3. When birds were 42 days old. the ileal villi were significantly longer ($p<0.05$) in birds given the medium MOS diet compared to the negative control. MOS treatments did not significantly affect the crypt depth of small intestine. but a tendency to reduce the crypt depth at the jejunum was observed at the early ages.

Dietary treatments affected the individual SCFA concentrations in the younger (21 days old) but not the older (42 days old) birds (Table 7). A significant increase ($p<0.05$) in propionate and butyrate concentration was observed in birds fed on the high MOS diet compared to the negative control or/and ZnB treatment, but total SCFA concentrations were not affected by the addition of MOS. Also MOS tended to increase the molar ratio of butyrate in 21-day-old birds and the molar ratio of propionate in 42-day-old birds compared to the negative control. In contrast. ZnB reduced ($p<0.05$) the molar ratio of butyrate in 42-day-old birds compared to the negative control.

DISCUSSION

As indicated by the growth performance results. MOS can positively affect the growth of birds. This information supports the findings that the addition of MOS to diets can improve the growth performance of broiler chickens (Hooge et al.. 2003; Kocher. 2004). Although there was no

Table 5. Effects of dietary treatments[1] on apparent jejunal and ileal protein and starch digestibility coefficient of broiler chickens on days 7, 21 and 42

Segment	NC	0.5 g/kg MOS	1 g/kg MOS	2 g/kg MOS	Stepdown MOS	ZnB	SEM
Jejunum							
Day 7[2]							
Protein	0.66	0.75	0.76	0.69	0.69	0.68	-
Starch	0.70	0.73	0.73	0.72	0.71	0.69	-
Day 21							
Protein	0.66	0.70	0.72	0.69	0.69	0.75	0.049
Starch	0.80	0.81	0.83	0.84	0.84	0.81	0.040
Day 42							
Protein	0.76	0.79	0.74	0.75	0.71	0.77	0.059
Starch	0.88	0.90	0.90	0.89	0.85	0.88	0.039
Ileum							
Day 7[2]							
Protein	0.85	0.84	0.85	0.79	0.80	0.84	-
Starch	0.95	0.96	0.96	0.95	0.96	0.97	-
Day 21							
Protein	0.75	0.79	0.81	0.83	0.82	0.79	0.034
Starch	0.99	0.98	0.99	0.99	0.99	0.99	0.03
Day 42							
Protein	0.80	0.82	0.85	0.86	0.84	0.82	0.029
Starch	0.98	0.98	0.99	0.96	0.97	0.98	0.016

[1] See Table 2.

[2] The samples from the same treatment were pooled for the determination of nutrient digestibility because of the little amount of digesta collected from one week old birds. Hence, one replicate per treatment was used.

Table 6. Effects of dietary treatments[1] on the gut morphometry of the small intestinal mucosa of broiler chickens on days 7, 21 and 42

Segment	NC	0.5 g/kg MOS	1 g/kg MOS	2 g/kg MOS	Stepdown MOS	ZnB	SEM
Jejunum							
Day 7							
Villus height (μm)	874^{bc}	904^{b}	984^{a}	980^{a}	959^{ab}	901^{b}	42.3
Crypt depth (μm)	124	125	113	105	109	126	8.5
Day 21							
Villus height (μm)	1,457^{b}	1,502^{ab}	1,560^{a}	1,553^{a}	1,548^{ab}	1,505^{ab}	59.4
Crypt depth (μm)	135	132	126	126	130	139	21.9
Day 42							
Villus height (μm)	1,650	1,591	1,659	1,659	1,672	1,695	121.2
Crypt depth (μm)	117	122	103	114	109	109	8.7
Ileum							
Day 7							
Villus height (μm)	565	549	531	542	540	538	42.7
Crypt depth (μm)	115	96	96	97	100	101	16.2
Day 21							
Villus height (μm)	748	778	723	780	788	789	72.4
Crypt depth (μm)	93	95	99	98	106	102	11.9
Day 42							
Villus height (μm)	855^{b}	922^{ab}	998^{a}	958^{ab}	979^{ab}	949^{ab}	71.5
Crypt depth (μm)	84	93	97	91	93	99	10

[1] See Table 2.

[a, b] Means within the same row with no common superscript differ significantly ($p<0.05$).

linearity in dose response to MOS inclusion, Tucker et al. (2003) studied the dose response of birds to MOS between 0 and 3 g/kg diet and found the optimal dose was around 1.5 g/kg. A relatively higher dosage level (2 g/kg MOS) was shown to be the optimum level in the current experiment, which is in agreement with the report by Kumprecht and Zobač (1997) that the optimum dosage of MOS added to broiler chickens diet is between 150 to 300 g per 100 kg feed.

One gram MOS per kg diet and step-down program are the dosage practice on some commercial farms. In the present study, birds fed on 1 g/kg MOS diet had a better

Table 7. Caecal volatile fatty acid results of broilers fed on the experimental diets[1] at different ages

	NC	0.5 g/kg MOS	1 g/kg MOS	2 g/kg MOS	Stepdown MOS	ZnB	SEM
Concentration (μmoles/ml content)							
Day 21							
Acetate	52.58	61.07	60.8	73.04	60.53	49.78	10.5
Propionate	5.83^{b}	6.84^{ab}	5.47^{b}	7.51^{a}	7.44^{ab}	5.47^{b}	1.27
Butyrate	10.36^{b}	11.46^{b}	10.48^{b}	16.42^{a}	12.19^{b}	9.70^{b}	3.54
Total VFA	76.62	82.46	80.85	100.52	83.09	70.57	22.8
Day 42							
Acetate	64.27	51.01	59.22	69.60	67.09	69.93	29.8
Propionate	3.72	3.43	6.00	6.02	4.93	6.30	2.77
Butyrate	12.98	9.08	9.41	11.29	11.03	7.66	6.23
Total VFA	83.27	70.22	74.24	90.11	85.50	88.86	37.8
Molar ratios of VFA (% of total VFA)							
Day 21							
Acetate	68.62	74.06	75.20	72.66	72.85	70.54	4.33
Propionate	7.61	8.29	6.77	7.47	8.95	7.75	2.94
Butyrate	13.52^{ab}	13.90^{ab}	12.96^{b}	16.34^{a}	14.67^{ab}	13.75^{ab}	11.7
Day 42							
Acetate	77.18	72.64	79.77	77.24	78.47	78.70	3.39
Propionate	4.47^{b}	4.88^{b}	8.08^{a}	6.68^{ab}	5.77^{ab}	7.09^{ab}	1.66
Butyrate	15.59^{a}	12.93^{ab}	12.68^{ab}	12.53^{ab}	12.90^{ab}	8.62^{b}	3.67

[1] See Table 2.

[a, b] Means within the same row with no common superscript differ significantly ($p<0.05$).

BWG in the last three weeks while birds fed on step-down MOS diet had a better FCR in the first three weeks than those on the negative control. However, an improvement in both BWG and FCR of birds was observed for 2 g/kg MOS treatment and the effects were comparable to ZnB treatment, especially in the first three-week feeding period.

It has been reported that birds give a greater response to increasing MOS supplementation from 1 to 21 d than in the 21-42 d period (Tucker et al., 2003; Jamroz et al., 2003). A similar trend was observed in the current experiment. These observations suggest that birds need a relatively high dosage level of MOS in early life. Early evaluation of the effects of antibiotics on the growth performance of birds showed similar results (Jukes, 1955). It is postulated that this is due to a less mature gut system, e.g. the differences in gut microflora population, in younger birds compared to older birds.

Young birds fed on MOS diets or ZnB diet had smaller (relative weight) liver than those birds fed on the negative control diet. The liver size is dependent on the gastrointestinal microflora and/or its fermentation products (Jozefiak et al., 2006). Reduced liver size was reported in germ-free vs. conventional rats and chickens (Wostman, 1981; Muramatsu et al., 1983).

The relative weight of the other visceral organs, including the small intestine, was not affected by MOS treatment, which is in agreement with the report by Iji et al. (2001) and Juskiewicz et al. (2003). However, the relative length of the small intestine of old birds seemed to be reduced in some MOS treatments compared to the negative control and a similar result was found on ZnB treatment. It has been reported that the length of the small intestine was generally shorter in the presence of antibiotics without any effects on digestive/absorptive function (Gordon, 1961a; Stutz et al., 1983).

No significant differences were found in the small intestinal digestibility of protein and starch between MOS treatment (s) and the negative control. This is in agreement with the findings of Alves et al. (2003). However, a numerically higher digestibility of nutrient was observed in young birds fed on medium, high MOS or ZnB diets than those birds in the negative control group but the effects disappeared when the birds got older, which might indicate a quick development of gut function by the addition of those in-feed additives. On the other hand, an improvement in the villus height of small intestine was observed in medium or high MOS treatment, which agrees with the reports by Spring (1996) and Iji et al. (2001). Similar results were observed with birds fed on ZnB diet.

However, MOS affected the caecal VFA profile of birds in a different way from ZnB. In general ZnB reduced the individual or total SCFA concentrations, but MOS increased propionate and/or butyrate concentration in the caeca of birds at different ages. In agreement with our findings, Santos et al. (2004) reported that propionic acid concentration was higher with MOS treatment when birds were raised on the re-utilized litter. A significant negative correlation was found between caecal propionic acid concentration and caecal *Salmonella* colonization with birds less than 10 days old (Nisbet et al., 1996), and Finucane et al. (1999) reported that 53% of *Salmonella* species tested possess type 1 fimbriae and they are supposed to be

adsorbed by MOS. However, it is not clear whether there is a direct relationship between MOS treatment and the caecal propionic acid concentration. On the other hand. MOS supplementation also increased the caecal butyrate concentration of birds. VFA. mainly butyrate. can exert a trophic effect not only on the local epithelial cells but also on jejunal structure (Lan et al., 2005).

The findings from this study suggest that the growth response of birds was more obvious to the high dosage level of MOS than the low or medium MOS or step down program. Mannanoligosaccharide and ZnB helped the gut development and maturation of younger birds compared to those in the negative control group. Mannanoligosaccharide showed some similar effects on the gut development of birds as those in ZnB group. however, it had a different effect on the caecal VFA profile.

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